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Haemato-biochemical studies on the haemoprotozoa and rickettsia induced reactive hepatopathy in animals

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Abstract

Haemato-biochemical changes in haemoprotozoa and rickettsia induced reactive hepatopathy in dogs and bovines were studied. Out of total 156 dogs presented in the clinics with hepatopathies, 65.38% were attributed to primary hepatitis and 34.62% to reactive hepatitis. Among dogs with clinical hepatopathy, 14.10% were diagnosed with haemoprotozoa/rickettsia infections viz. *Babesia gibsoni* (22.2%), *Ehrlichia canis* (14.81%) and mixed infection with *Babesia gibsoni* and *Ehrlichia canis* (3.7%). Age wise distribution revealed age group of 2 to <4 years and <2 years had higher incidence of haemoprotozoan induced reactive hepatitis. In bovines, 39.13% cases were diagnosed with reactive hepatitis out of which 23.19% were due to haemoprotozoa/rickettsial infections with *Babesia bigemina*, *Theileria annulata* and *Anaplasma marginale*. Age wise distribution revealed age group of 2 to<4 years and 4 to <6 years had higher incidence of haemoprotozoan induced reactive hepatitis. Haematological studies in dogs revealed significant (P<0.01) decrease in Hb, PCV, TEC values and platelet counts and increase in MCV, MCHC and TLC values in affected animals compared to the healthy control. Biochemical studies revealed significant increase in ALT,AST, ALP, GGT values along with increase in total bilirubin indicating hepatopathy. In cattle, haematological studies revealed significant decrease in Hb, PCV, TEC values with neutrophilia, lymphocytosis and monocytosis. A significant increase in AST, ALP, GGT and total bilirubin and BUN values was recorded.

Key words: Anaplasma, Babesia, biochemical, cattle, dog, Ehrlichia, haematology, Theileria, reactive hepatopathy

Haemoprotozoan diseases such as *Babesiosis, Theileriosis* and *Anaplasmosis* are tick borne protozoan infections of cattle and buffaloes. Babesiosis in cattle is caused by intraerythrocytic protozoan parasite, *Babesia bigemina*, and is characterized by haemolytic anaemia and fever, with occasional haemoglobinuria and death (Ristic 1981). Tropical theileriosis caused by the protozoan parasite, *Theileria annulata* is very severe in cattle in endemic areas and causes a lot of economic losses due to mortality and loss of productivity from cattle (Lotfollahzadeh *et al*. 2010). Anaplasmosis, formerly known as gall sickness is a tick-borne disease caused by an obligate intraerythrocytic rickettsial microorganism, *Anaplasma marginale* and *Anaplasma centrale* of the order *Rickettsiales*, family *Anaplasma taceae*. Disease is mainly characterized by progressive haemolytic anaemia associated with fever, jaundice, decreased milk production, abortions, hyperexcitability and in some cases sudden death (Kumar *et al*. 2015).

Canine babesiosis is a common tick transmitted disease of dogs worldwide. Infections in dogs can cause considerable morbidity and mortality. Dogs typically present with the acute, severe form of babesiosis which is characterized by findings such as abnormally dark urine, fever, weakness, pale mucous membranes, depression, swollen lymph nodes, and an enlarged spleen. Clinical signs are generally attributed to haemolysis caused by the organisms in the

erythrocytes but in some animals with some *Babesia* spp. there can be an immune mediated component to the anaemia and/or a severe inflammatory reaction associated (Koster *et al*. 2015; Brahma *et al*. 2019).

The infection with haemoparasites in cattle can cause increased serum activities of liver enzymes like AST and ALP indicative of the hepatic injuries and can cause haemoprotozoan associated hepatopathies (Lotfollahzadeh *et al*. 2011; Shah *et al*. 2016; Primus *et al*. 2018; Alekish and Ismail 2019). Similarly, in dogs, complicated babesiosis involves clinical manifestations that are not related to haemolytic disease such as coagulopathy, icterus and hepatopathy, haemoconcentration, immune-mediated haemolytic anaemia (IMHA), shock etc. (Vishwakarma and Nandini 2019). A severe hepatic disease has been reported uncommonly in pancytopenic dogs with advanced *E. canis*-induced BM aplasia (Mylonakis *et al*. 2010).

The present study was done to evaluate the haemato-biochemical alterations in haemoprotozoa induced reactive hepatopathy in cattle and dogs in order to assess whether the liver enzyme assays could be useful as diagnostics for haemoprotozoa induced reactive hepatopathies.

Materials and Methods

A total of 156 dogs, 28 cattle and 18 buffaloes with symptoms and manifestations of hepatic dysfunction like (anorexia, fatigue, exercise intolerance, jaundice, ascites, cachexia, seizures etc.) presented in the Veterinary Clinics, CSKHPKV, Palampur were selected for the study. Clinical parameters such as rectal temperature, heart rate and respiration rates were recorded on the day of presentation to the clinics. They were further classified as patients with primary hepatitis and reactive hepatitis based on the laboratory findings (haemato-biochemical tests and screening for haemoprotozoa by standard laboratory techniques) as described below. The dogs of both sexes (n=54) with reactive hepatitis falling into age range 3 months to 14 years were divided into age groups viz.<2years, 2 to <4 years, 4 to <6years,6 years to <8 years, 8 to <10 years and >10 years. Similarly, cattle (n=12) and buffalo (n=6) of both sexes with reactive hepatitis falling into age range 1.2 months to 8.5 years were divided into age groups viz.<2years, 2 to <4 years, 4 to <6years,6 years to <8 years, 8 to <10 years and >10 years for further studies on etiology. Twelve clinically healthy dogs and 6 clinically healthy bovines were also included in the study as healthy control.

Haemato-Biochemical assays

For haematological examination (complete blood count) and haemoprotozoan detection, approximately 2 ml of blood was collected as per standard protocol aseptically in sterile plastic vials containing tri-potassium salt of ethylenediamine-tetra acetic acid (K_3EDTA, 3.6mg/2ml) (HemoTube™, MB Lab Consumables, Sec. 83 Noida, UP). The Complete blood count (CBC) was assessed using an Auto-Haematology Analyser BC-2800 Vet (Manufactured by Mindray Medical International Limited, Shenzhen-China; Marketed by Fresenius Medical Care Private Limited, New Delhi). Parameters assessed were: red blood cell count (RBC), hemoglobin (Hb), PCV, PLT count, white blood cell count (WBC), WBC differential count including neutrophils, lymphocytes, monocytes, eosinophil.

Heparinized syringe was used to collect approximately another 4 ml blood for taking out plasma. Plasma for biochemical analyses were removed by centrifugation for the measurement of activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and Gamma glutamyl transferase (GGT). Activities were expressed in units/liter of plasma. Similarly, Total Protein, albumin, globulin, A/G ratio was estimated and were expressed in g/dL of plasma. Total bilirubin, Blood Glucose, BUN and Creatinine levels were also estimated. Commercial reagent kits, based on spectrophotometric methods, were used to assay these parameters through semi-automatic biochemical analyser Microlab 300 Clinical Chemistry Analyser (by Merck Limited, Mumbai). The results were compared with values obtained from healthy control.

Diagnosis of haemoprotozoa

Blood was collected, combined with an anticoagulant and smeared on a glass slide, air-dried, fixed with methanol, and stained with Giemsa. The slide was then washed thoroughly and dried. Intraerythrocytic parasites are observed under a microscope using a 100X objective and a drop of immersion oil (morzaria) for presence of *A.*

marginale, Theileria spp., and *Babesia* spp. in erythrocytes and *Ehrlichia* in monocytes. Examination of the smears was performed at 100× magnification with compound microscope by searching at least 50 fields per slide. The parasites were identified as described by OIE 2008.

Statistical analysis

The data obtained were subjected to statistical analysis by using computer software Instat from Graphpad software, 2008. The mean values of different parameters between control and diseased group were compared at 1% and 5% level of significance employing t- test and ANOVA.

Results and Discussion

After definitive diagnosis about etiology, 22 dogs and 11 bovines were found to be affected from haemoprotozoa/rickettsia induced hepatopathy. The affected dogs exhibited significantly ($P<0.01$) increased heart rate and rectal temperature ($P<0.05$) (Table 1). In bovines, there was a significant ($P<0.05$) rise in heart rate, rectal temperature and respiration rate (Table 2). Out of total 156 dogs presented in the clinics with hepatopathies, 65.38% were attributed to primary hepatitis and 34.62% to reactive hepatitis (Table 3). Out of all the cases, 14.10% were diagnosed with haemoprotozoa/rickettsia infections with *Babesia gibsoni* (22.2%), *Ehrlichia* canis (14.81%) and mixed infection with *Babesia gibsoni* and *Ehrlichia canis* (3.7%). Age wise distribution revealed that age group of 2 to <4 years and <2 years had a higher incidence of haemoprotozoan induced reactive hepatitis (Table 5). In bovines, 39.13% cases were diagnosed with reactive hepatitis out of which 23.19% were attributed to haemoprotozoa/rickettsial infections with *Babesia bigemina, Theileria annulata* and *Anaplasma marginale* (Table 4). Age wise distribution revealed age group of 2 to <4 years and 4 to <6 years had a higher incidence of haemoprotozoan induced reactive hepatitis (Table 6). The haemoprotozoan infections in canines and bovines have been commonly reported in India and globally (Varshney *et al*. 2009, Karunakaran *et al*. 2011, Tresamol *et al*. 2013, Jain *et al*. 2017 and Kaur *et al*. 2021).

Table 1. Mean values of vital body parameters in dogs with reactive hepatic disorders

Parameters	Healthy Control (n=12)	Haemoprotozoan/ Rickettsial diseases (n=22)
Rectal Temperature (^{0}F)	101.53 ± 0.21	103.03±0.58*
Heart rate (per min.)	78.71 ± 2.28	129.87±3.58**
Respiration rate (per min.)	30.42 ± 2.49	33.52±4.85

Table 2. Mean values of vital body parameters in bovines with reactive hepatic disorders

Parameters	Healthy Control (n=6)	Haemoprotozoan/ Rickettsial diseases (n=11)
Rectal Temperature (^{0}F)	101.12 ± 0.24	102.21±0.40*
Heart rate (per min.)	70.50 ± 1.72	91.33±7.49*
Respiration rate (per min.)	20.86 ± 2.08	34.85±4.38*

* Significant at 5% ($P<0.05$); ** Significant at 1% ($P<0.01$)

Table 3. Distribution of cases of hepatic disorder in dogs

Condition	Total no. of cases	% out of total cases
Primary Hepatitis	102	(65.38%)
Reactive Hepatitis	54	(34.62%)
Total	**156**	
Reactive Hepatic Diseases:	**54**	
Haemoprotozoan/ Rickettsial diseases	22 (40.74%)	14.10%
Babesia gibsoni	12 (22.22%)	
Ehrlichia canis	8 (14.81%)	
Mixed infection of *B. gibsoni* and *E. canis*	2 (3.70%)	

Table 4. Distribution of cases of hepatic disorder in bovines

Condition	Total no. of cases	% out of total cases	Species wise Distribution
Primary hepatopathies:	28	60.87%	Cattle:16 Buffalo:12
Reactive hepatopathies:	18	39.13%	Cattle: 12 Buffalo:6
Total	**46**		
Reactive hepatopathies:			
Haemoprotozoan/ Rickettsial diseases	11	23.91%	Cattle=8 Buffalo=3
Babesiosis	2		Cattle:2 Buffalo:Nil
Theileriosis	3		Cattle:2 Buffalo :1
Anaplasmosis	4		Cattle : 2 Buffalo :2
Combined infection of Theileria and Anaplasma	2		Cattle : 2 Buffalo :Nil

Table 5. Age and sex wise distribution of canine cases suffering from haemoprotozoan/ rickettsia induced reactive hepatic disorders

Condition	Age group						Total Males	Total Females	Ag Range
	<2 years	2— <4 years	4— <6 years	6— <8 years	8— <10 years	> 10 years			
Haemoprotozoan/ Rickettsial diseases (n=22)	10	6	3	2	1	Nil	18	4	3m-8Y

Table 6. Age and sex wise distribution of bovine cases suffering from haemoprotozoan/ rickettsia induced reactive hepatic disorders

Condition	Age group						Total Males	Total Females	Age Range
	<2 years	2— <4 years	4— <6 years	6— <8 years	8— <10 years	> 10 years			
Haemoprotozoan/ Rickettsial diseases (n=11)	Nil	6	3	2	Nil	Nil	1	10	2-6 Y

Haematological studies in dogs revealed significant (P<0.01) decrease in Hb, PCV, TEC values and platelet counts and increase in MCV, MCHC and TLC values in haemoprotozoa induced reactive hepatitis compared to the healthy control (Table7). Biochemical studies revealed a significant increase (P<0.01) in ALT, AST, ALP and GGT values indicating hepatopathy and a significant increase (P<0.01) in total bilirubin (Table 8). Similarly, Showkat *et al*. (2011) reported that *Babesia* infection in dogs caused anaemia and thrombocytopenia. Bilwal *et al*. (2018) found that the mean values of ALP, ALT, AST, TB and A:G in infected dogs were significantly higher than healthy control group. These abnormalities resulted probably due to liver damage in infected anaemic dogs. Severe symptomatic hepatitis as the predominant clinical manifestation has been reported in pancytopenic dogs with advanced *Ehrlichia canis* induced aplasia and acute monocytic Ehrlichiosis (Mylonakis 2010). However, in another study by Zygner *et al*. (2011), the differences in the activities of the enzymes were not statistically significant and they suggested that mild anaemia, as the only factor, has no influence on ALT, AST, or ALP activity in canine babesiosis. However, this study certainly cannot exclude the possibility that a more severe anaemia can have a major effect on the liver.

In cattle, haematological studies revealed that there was a significant (P<0.01) decrease in Hb, PCV, TEC values and significant (P<0.01) increase in

Table 7. Meanvalues of haematological parameters in dogs with reactive hepatic disorders

Parameters	Healthy Control (n=12)	Haemoprotozoan/ Rickettsial diseases (n=22)
Hb (g/dL)	13.42 ± 0.41	7.64±1.04**
PCV (%)	42.17 ± 1.23	24.49±2.92**
TEC ($\times 10^{12}$/L)	6.58 ± 0.22	3.18±0.55**
TLC ($\times 10^{9}$/L)	10.42 ± 0.61	29.43±6.11**
N (%)	67.83 ± 1.76	71.10±3.71
L (%)	26.70 ± 2.18	23.91±3.63
M (%)	2.83 ± 0.38	3.43±0.31
E (%)	1.85 ± 0.29	1.05±0.18*
B (%)	0.79 ± 0.21	0.52±0.38
MCV (fL)	63.95 ± 0.76	83.28±4.80**
MCH (pg)	20.36 ± 0.41	24.63±1.05**
MCHC(g/dL)	32.03 ± 0.69	29.96±0.79
Platelets ($\times 10^{9}$/L)	304.70 ± 21.37	98.23±19.61**

* Significant at 5% (P<0.05); ** Significant at 1% (P<0.01)

Table 8. Mean values of plasma biochemical parameters in dogs with reactive hepatic disorders

Parameters	Healthy Control (n=12)	Haemoprotozoan/ Rickettsial diseases (n=22)
ALT (U/L)	24.77 ± 3.83	89.30±12.28**
AST (U/L)	33.04 ± 3.69	74.65±9. 72**
ALP (U/L)	62.75 ± 6.18	217.60±20.36**
GGT (U/L)	2.65 ± 0.48	10.33±3.21*
TP (g/dL)	6.59 ± 0.23	6.14±0.38
Albumin (g/dL)	3.55 ± 0.20	3.21±0.32
Globulin (g/dL)	3.04 ± 0.28	2.94±0.30
A/G ratio	1.22 ± 0.17	1.09±0.18
Total bilirubin (mg/dL)	0.39 ± 0.07	4.82±1.02**
Direct bilirubin (mg/dL)	0.15 ± 0.03	1.86±0.47**
Indirect bilirubin (mg/dL)	0.24 ± 0.05	2.96±0.54**
Glucose (mg/dL)	93.91 ± 3.83	103.56±12.66
BUN (mg/dL)	17.56 ± 1.31	28.20±8.15
Creatinine (mg/dL)	0.82 ± 0.08	0.86±0.32

* Significant at 5% (P<0.05); **Significant at 1% (P<0.01)

MCHC with neutrophilia, lymphocytosis and monocytosis in haemoprotozoa induced reactive hepatitis as compared to the healthy control (Table 9). A significant (P<0.05) increase in AST, ALP, GGT and total bilirubin and BUN (P<0.01) values was recorded indicating hepatopathy and nephropathy (Table10). The elevated hepatic transaminase levels (*i.e.*aspartate aminotransferase [AST] and alanine aminotransferase [ALT], an elevated alkaline phosphatase level, and hyperbilirubinemia are variably present in infection with haemoprotozoan parasites such as *Babesia* sp. (Primus *et al*. 2018). An analysis for ALT, AST, ALP activities were carried out in infected and uninfected cattle by Lotfullazahed *et al*. (2011) in order to determine the degree of hepatic damage and blood iron status caused by *Theileria annulata* and *Babesia bigemina* and they reported increased serum AST and ALP activities in infected cattle which indicated the hepatic injuries associated with infection with *T. annulata* and *B. bigemina*. The increased level of AST in bovine babesiosis indicates hepatic damage and also haemolytic anaemia because this enzyme exists not

Table 9. Mean values of haematological parameters in bovines with reactive hepatic disorders

Parameters	Healthy Control (n=6)	Haemoprotozoan/ Rickettsial diseases (n=11)
Hb (g/dL)	11.15 ± 0.35	6.18±0.88**
PCV (%)	34.25 ± 1.20	22.46±3.07**
TEC ($\times 10^{12}$/L)	6.91 ± 0.18	4.29±0.65**
TLC ($\times 10^{9}$/L)	7.88 ± 0.41	11.40±1.93
N (%)	28.60 ± 1.28	52.80±6.11**
L (%)	64.39 ± 0.82	36.15±6.31**
M (%)	3.10 ± 0.33	8.65±1.53**
E (%)	3.45 ± 0.47	2.10±0.57
B (%)	0.46 ± 0.20	0.35±0.22
MCV (fL)	49.18 ± 0.83	54.21±3.26
MCH (pg)	16.10 ± 0.26	14.46±0.80
MCHC(g/dL)	32.48 ± 0.48	25.43±1.61**
Platelets ($\times 10^{9}$/L)	351.80 ± 20.41	373.38±54.68

* Significant at 5% (P<0.05); ** Significant at 1% (P<0.01)

Table 10. Mean values of plasma biochemical parameters in bovines with reactive hepatic disorders

Parameters	Healthy Control (n=6)	Haemoprotozoan/ Rickettsial diseases (n=11)
ALT (U/L)	36.10 ± 3.04	49.60±12.10
AST (U/L)	67.30 ± 3.96	211.42±26.38**
ALP (U/L)	49.60 ± 4.82	204.67±58.11*
GGT (U/L)	9.35 ± 0.73	27.60±6.72*
TP (g/dL)	6.89 ± 0.25	6.04±0.42
Albumin (g/dL)	3.06 ± 0.26	2.65±0.15
Globulin (g/dL)	3.83 ± 0.29	3.39±0.11
A/G ratio	0.82 ± 0.05	0.78±0.06
Total bilirubin (mg/dL)	0.34 ± 0.07	1.68±0.32**
Direct bilirubin (mg/dL)	0.23 ± 0.03	0.95±0.30*
Indirect bilirubin (mg/dL)	0.11 ± 0.01	0.73±0.22*
Glucose (mg/dL)	56.67 ± 1.98	81.33±9.36*
BUN (mg/dL)	15.39 ± 0.87	29.12±4.22**
Creatinine (mg/dL)	1.03 ± 0.21	1.17±0.12

* Significant at 5% (P<0.05); ** Significant at 1% (P<0.01)

only in the liver, but also in the heart, kidney, skeletal muscle, and erythrocytes (Boyd 1962). Elevation of enzyme activity in serum indicates necrosis or disease in liver or muscle during tropical theileriosis. *Theileria annulata* infection causes coagulative necrosis of hepatocytes, distortion of the hepatic cord, and lymphocyte infiltration into the periportal regions indicating severe damage to the hepatobiliary system due to hypoxia resulting from anemia and jaundice (Sandhu *et al*.1988). Primus *et al*. (2018) showed that direct hyperbilirubinemia as well as persistently elevated INR (International Normalized Ratio) and decrease in albumin were the result of fulminant liver failure which occurred as a complication of babesiosis and demonstrated the usefulness of AST and ALT as prescreening enzymatic assays for babesiosis. The reason could be hepatocellular injury secondary to oxidative stress caused by host immune response against the parasite (Shah *et al*. 2018). Nassar and Seth (2017) reported the case of an elderly man with acute

liver failure due to an infection with *Babesia microti*. In this case, the diagnosis remained unclear given the progressive elevation of bilirubin as well as the increase in aspartate aminotransferase and alanine aminotransferase. Fujinaga (1981) found a significant rise in GOT (AST) level in cattle experimentally infected with *Babesia ovata* during the hemolytic phase of the disease as compared with that in theuninfected control group. Similarly, Alikish and Ismail *et al*. (2020) have reported elevated levels of liver enzymes in cattle with anaplasmosis.

Conclusion

The infection with haemoparasites in cattle and dogs can cause increased serum activities of liver enzymes like AST and ALP and can cause haemoprotozoan associated hepatopathies. The change in haemato-biochemical parameters during haemoprotozoan/ rickettsia induced reactive hepatitis assay could be very useful as prescreening assays, however, a caveat should be considered that these enzymes are associated with liver malfunction and increase in their activities can be due to other infectious diseases and even non-infectious diseases such as fatty liver disease.

Conflict of interest: The authors declare that there is no conflict of interest among the authors in this research paper.

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